

Materials List for:

Antibody-Free Assay for RNA Methyltransferase Activity Analysis

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Materials

Name	Company	Catalog Number	Comments
10 bp DNA Ladder	Invitrogen	10821-015	10 bp DNA Ladder kit.
10% Ammonium Persulfate (APS)	N/A	N/A	For urea denaturing polyacrylamide gel (For 10 mL, dissolve 1g in 8 mL of milliQ water; adjust volume to 10 mL with milliQ water; filter the solution using a 10 mL syringe equipped with a 0.45 µm filter).
10X TBE Buffer	N/A	N/A	For urea denaturing polyacrylamide gel (For 1L, add 108 g of Tris Base, 55 g of Boric Acid to a cylinder with a stir bar; add 800 mL of distilled water and let dissolve; add 40mL of 0.5 M Na2EDTA (pH 8.0); adjust volume to 1L with milliQ water; filter the solution using a 0.22µm filter).
10X TBS	N/A	N/A	For 1L, add 60.5 g of Tris Base, 87.6 g of NaCl to a cylinder with a stir bar; add 800 mL of distilled water and let dissolve; adjust pH to 7.5 with concentrated HCl; adjust volume to 1L with milliQ water; filter the solution using a 0.22 µm filter.
Acrylamide: Bis-Acrylamide 29:1 (40% Solution/Electrophoresis), Fisher BioReagents	Fisher	BP1408-1	For urea denaturing polyacrylamide gel.
ADENOSYL-L-METHIONINE, S-[METHYL-3H]; (SAM[3H])	Perkin Elmer	NET155V250UC	For in vitro methylation of RNA; Concentration = 1.0 mCi/mL; Specific activity = 17.1 Ci/mmol; Molarity= (1.0 Ci/L)/(83.2 Ci/mmol) = 0.0584 mmol/L = 58.4 µM. Upon receipt of the frozen 3H-SAM tube, thaw it at 4°C, make 20 µL aliquots, and freeze them at -30°C. Never refreeze and reuse a partially used aliquot.
Amersham Hypercassette Autoradiography Cassettes	GE Healthcare	RPN11649	For autoradiogram gel exposure.
Amersham Hyperfilm MP	GE Healthcare	28906846	For autoradiogram gel exposure.
Beckman Scintillation Counter	Beckman	LS6500	For liquid scintillation count.
Biorad Mini Horizontal Electrophoresis System	Biorad	1704466	Mini Horizontal Electrophoresis System.
cOmplete Mini EDTA-free Protease Inhibitor Cocktail Tablets	Roche Applied Science	4693159001	For a 20X solution, dissolve 1 tablet in 0.525 mL of nuclease free water.

Criterion Cell	Biorad	345-9902	RNase free empty cassette for polyacrylamide gel.
Criterion empty Cassettes	Biorad	1656001	Vertical midi-format electrophoresis cell.
DeNovix DS-11 Microvolume Spectrophotometer	DeNovix	DS-11-S	Microvolume Spectrophotometer for measuring DNA and RNA concentration.
Ecoscint Original	National Diagnostics	LS-271	For liquid scintillation count.
Fisherbrand 7mL HDPE Scintillation Vials	Fisher	03-337-1	For liquid scintillation count.
Fluoro-Hance-Quick Acting Autoradiography Enhancer	RPI CORP	112600	For autoradiogram gel pretreatment.
Gel dryer	Biorad	1651745	For drying gel.
Gel Loading Buffer II	Ambion	AM8547	For loading RNA in denaturing polyacrylamide urea gel (composition: 95% Formamide, 18 mM EDTA, and 0.025% SDS, Xylene Cyanol, and Bromophenol Blue).
GeneCatcher disposable gel excision tips	Gel Company	NC9431993	For removing bands from agarose and polyacrylamide gels.
Megascript Kit	Ambion	AM1333	For in vitro transcription with T7 RNA polymerase.
Perfectwestern Extralarge Container	Genhunter Corporation	NC9226382 (clear)/ NC9965364 (black)	Gel staining box.
pRZ	Addgene	#27663	Plasmid for producing in vitro transcripts with homogeneous ends
Qiagen RNeasy MinElute Cleanup	Qiagen	74204	For RNA clean-up, use modified protocol provided in the protocol.
QIAquick Gel Extraction Kit (50)	Qiagen	28704	Kit for gel extraction and clean up of dsDNA fragment used for in vitro transcription.
Saran Premium Plastic Wrap	Saran Wrap	Amazon	For drying gel.
SYBR Gold	Invitrogen	S11494	Ultra sensitive nucleic acid gel stain.
SYBR Safe	Invitrogen	S33102	Nucleic acid gel stain.
TE	Sigma	93283-100ML	10 mM Tris-HCl, 1 mM disodium EDTA, pH 8.0
TEMED	Fisher	110-18-9	For urea denaturing polyacrylamide gel.
Thermomixer with SMARTBLOCK 24X 1.5mL TUBES	eppendorf	5382000023/5361000038	For temperature controlled incubation of 1.5 mL tubes.
TOPO TA Cloning Kit	life technologies		Kits for fast cloning of Taq polymerase-amplified PCR products into vectors containing T7 and/or SP6 promoters for in vitro RNA transcription.
TURBO DNase (2 U/μL)	Ambion	AM2238	For DNA removal from in vitro transcription reactions.
Urea	Sigma	51456-500G	For urea denaturing polyacrylamide gel.
Whatman 3MM paper	GE Healthcare	3030-154	Chromatography paper for drying gel.